Toxoplasma gondii

***Toxoplasma gondii* (*T.gondii*),** Is a protozoan parasite widely distributed around the world.It has been estimated that up to third of the world's population is infected by *T. gondii* .The word Toxoplasma originated from the Greek world toxon , which mean "bow " and plasmid mean "form". The original Greek meaning is the one used for the word Toxoplasma, meaning "bow" shaped organisms. Showed figure 2 This organism was first described in 1908 in Tunis by Nicolle and Manceaux within the tissues of the gondii (Ctenodoactylus *gondii*).In the same year it was also described in Brazil by splendor within the tissues of a rabbit

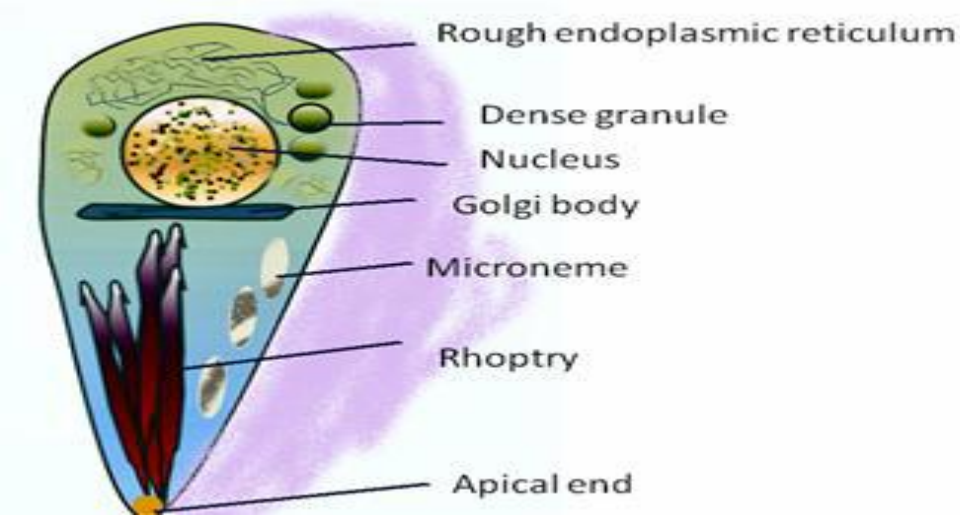

Figure 2. diagram of *T.gondii*

Classification*:*

The following classification has been cited in NCBI Taxonomy

Database

Domain: Eukarya; *T. gondii* is a single celled organism that contains a nucleus and has membrane bound organelles.

Kingdom: Alveolata: this group contains protozoa that was found to have a common ancestor 900 Million years ago. This precursor had structures that are now homologous between all members of this group.

Phylum: Apicomplexa; members of this group have highly developed structures at their anterior regions called Opical complexes, which are used in host cell invasion.

Class: coccidia, members of this group are obligating intra cellular Parasite .They generally complete the sexual stage in their life cycles within a host's intestinal tract.

Order: Eucoccidiorida, member of this group contain parasites of human, domesticated animals, wild animals, and birds.

Family: Sarcocystidae: These group members carry out a life cycle that requires more than one obligatory host. The hosts themselves usually participate in a predatory prey/ relationship. Oocysts are passed between them through feces.

Genus: Toxoplasma; this genus requires transmission between a member of the felidae and the rodents to carry out its sexual life cycle.

Species: *Toxoplasma gondii*; this is the only species in the genus Toxoplasma

Geographic Distribution*:*

The protozoan parasite *Toxoplasma gondii* infects more than a billion people worldwide].Serologic prevalence data indicate that toxoplasmosis is the one of the most common of human infections throughout the world. Infection is more common in warm climates and at lower altitudes than in cold climates and mountainous regions .High prevalence of infection in France has been related to a preference for eating raw or under cooked meat, while high prevalence in central America has been related to the frequency of stray cats in a climate favoring survival of Oocystes

Montoya et al showed The prevalence of *T. gondii* infection in the general population of several countries including France, Belgium, and the United Kingdom and the incidence of congenital toxoplasmosis have been reported to be decreasing over the past few decades.

Morphology*:*

T.gondii occurs in three forms; trophozoite, tissue cyst and oocyst.

The trophoziote and tissue cyst represent stages in asexual multiplication (Schizogony). While the oocyst is formed by sexual reproduction (gametogony or Sporogony).

All three forms occur in the domestic cat and other felines which is the definitive host and which support both schizogony and gametogony.

Only the sexual forms, trophoziotes and tissue cysts are present in other animals, including humans and birds, which are the intermediate hosts. Both oocyst and tissue cysts are infective by ingestion.

Trophoziote *:*

The trophozoite is crescent – shaped, with one end pointed and the other end rounded. It is measured approximately 2-3 μm by 4-7 μm. Showed figure 2.1. The nucleuse is ovoid and situated near the blunt end of the parasite.

It can invade any nucleated cell and replicate within cytoplasmic vacuoles by a processes called endodygony or internal budding – daughter trophoziote being formed, each surrounded by its own membrane .The term tachyzoite (tachos = speed in Greek) was coined by frenkel, to describe the stage that rapidly

multiplied in any cell of the intermediate host and in nonintestinal epithelial cells of the definitive host. The term tachyziote replaces the previously used term trophoziote (trophicos = feeding in Greek). Tachyziote have also been termed endodyzoites or endozoites. Aggregates of numerous tachyziotes are called clones, terminal colonies, or groups.

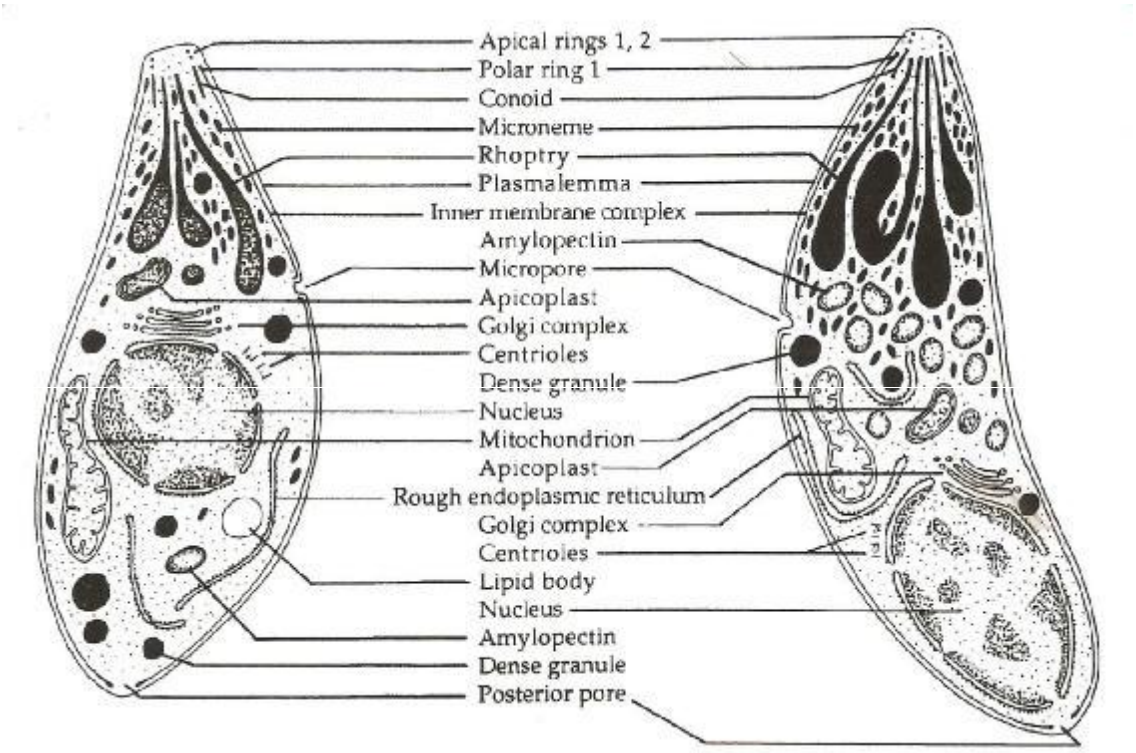

Figure 2.1 the tachyziote (left) and a bradyziote (right) of *T.gondii*

Tissue cysts *:*

The tissue cysts is formed during the chronic phase of the infection and can be found in the muscle and various other tissues and organs , include the Brain the term bradyzoites (Brady = slow in Greek) was also coined by Frekel , to describe the organism multiplying slowly within a tissue cysts . Bradyzoite are also called cystziotes. Tissue cysts grow and remain intra cellular, as the bradyziote divide by endo-dyogony **.** Tissue cyst vary in size, young tissue cyst may be as small as 5 μm in diameter and contain only two bradyzoite while older ones may contain hundreds of organisms. As in figure 2.2. Bradyziote differ structurally only slightly from tachyziote. They have situated toward the posterior end whereas the nucleus in tachyzoite is more centrally located

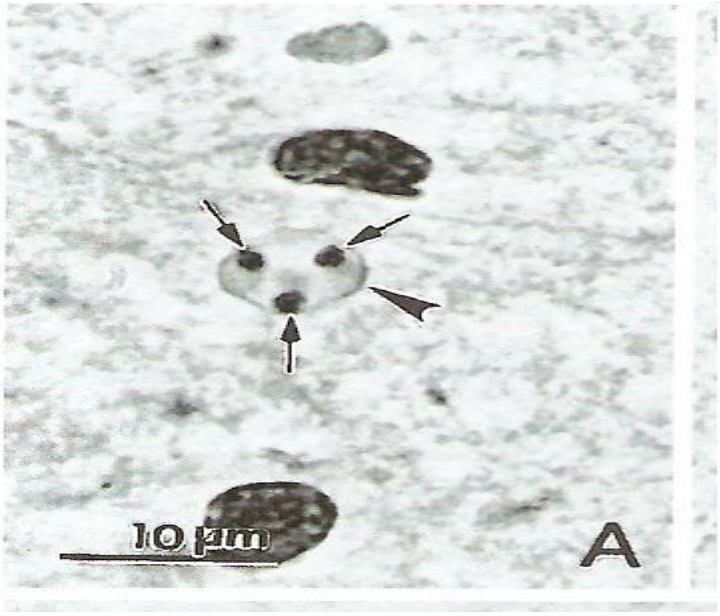

Figure 2.2 the tissue cyst of *T.gondii* in mouse brain

Oocysts:

Oocystes develop only in definitive hosts – in the intestine of cats and other felines when cats get infected by ingestion of either tissue cysts or Oocysts. The parasites develop in the intestinal epithelial cells, where both Schizogony and gametogony take place. Male and female gametocytes develop and after Fertilization, the zygote gets surrounded by a thin, but extremely resistance wall. This is Oocyst, which is sepherical or ovoid, about 10 to 12 µm in size and contains a sporoblast. Showed figure 2.3.

Cast shed millions of oocysts per day in faeces for about two weeks during the primary infection. The freshly passed Oocyst is not infections. It becomes infections only after development in soil or in water for a few days. During this state of sporulation, the sporoblast divides in two sporcysts and four sporozoite is the infective from. Showed figure 2.4. It is very resistance to environmental conditions and can remain infective in soil for about a year. When the infective Oocyst is ingested it releases sporozoites in the intestine, which initiate infection [24].

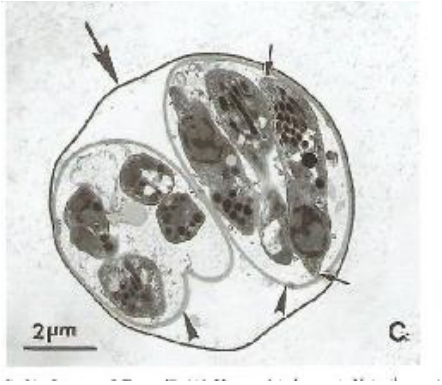

Figure 2.3 the Oocyst of *T.gondii*

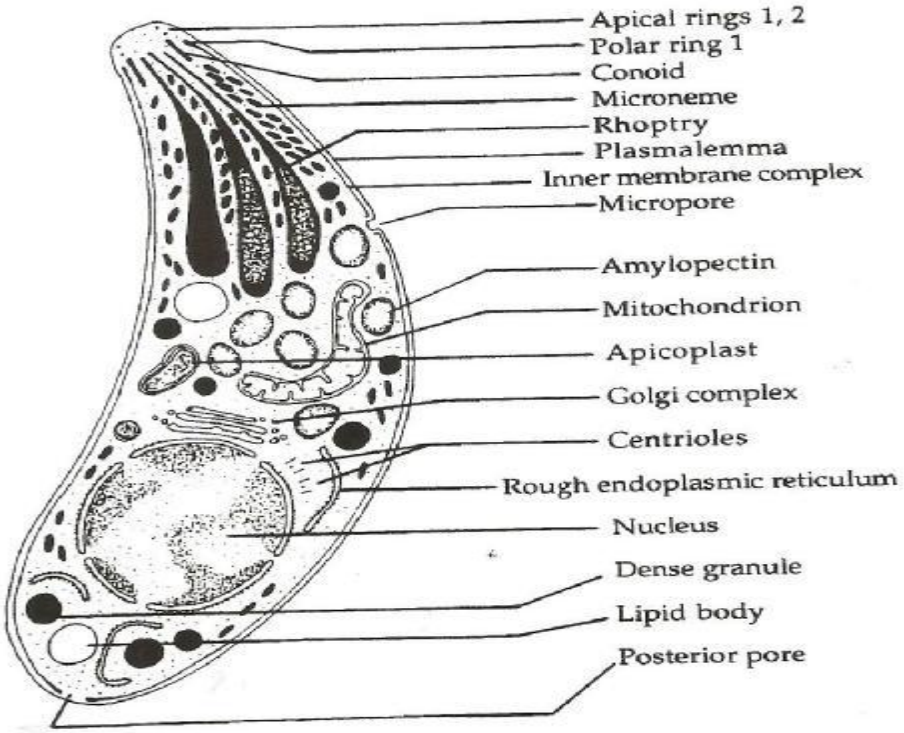

Figure 2.4 the sporozoite of *T.gondii*

Life Cycle

The life cycle of *T. gondii* consists of two stages asexual and sexual: as in figure 2.5. This asexual stage takes place in the intermediate hosts, which are mammals or bird. During this phase rapid intracellular growth of the parasite as tachyziote takes place (generation time in vitro is 6 – 8 hr). The oval or crescent – shaped tachyziotes can infect and multiply in almost any nucleated mammalian or avian cell . Following accumulation (64 – 128), tachyziotes are secreated into the blood stream , and spread in the body, leading to development of an acute disease (parasitemia). The normal immune response and transformation of the tachyziote into cyst formation bradyziote limit the acute stage and establish a chronic infection. Bradyziotes differ from tachyziotes mainly in their extremely slow multiplication rate (their name reflects this slow process), and in the distinct set of proteins they express. The cysts are formed mainly in neural and muscular tissues especially brain, skeletal and cardiac muscles and can persist, inactivated, in the body for a very long time. In the immunocompromised patient the release of bradyziotes from the cyst, may cause acute encephalitis.

The sexual stage takes place in intestine of the definitive host. Known definitive hosts are members of the feline family, predominantly domestic cast. When bradyziotes or Oocysts are ingested by a feline, formation of Oocysts processed in the epithelium of the small intestine. Several million unsporulated Oocystes may be released in the feces of a single cat over a period 3 – 18 days, depending on the stage of *T. gondii* ingested . Under mild environmental conditions Oocysts may sporulate. Within a 3 week period . Those infecting humans and other intermediate hosts. Oocyst can spread in the environmental and contaminate

water, soil, fruits, vegetables and herbivores following consumption of infected plan material. Investigation of outbreak of toxoplasmosis have led to recovery of Oocystes from soil , but not from water .

Oocystes have been found to be very stable, especially in warm and humid environment, and resistant to many disinfecting agents [, but survive poorly in arid, cold climates

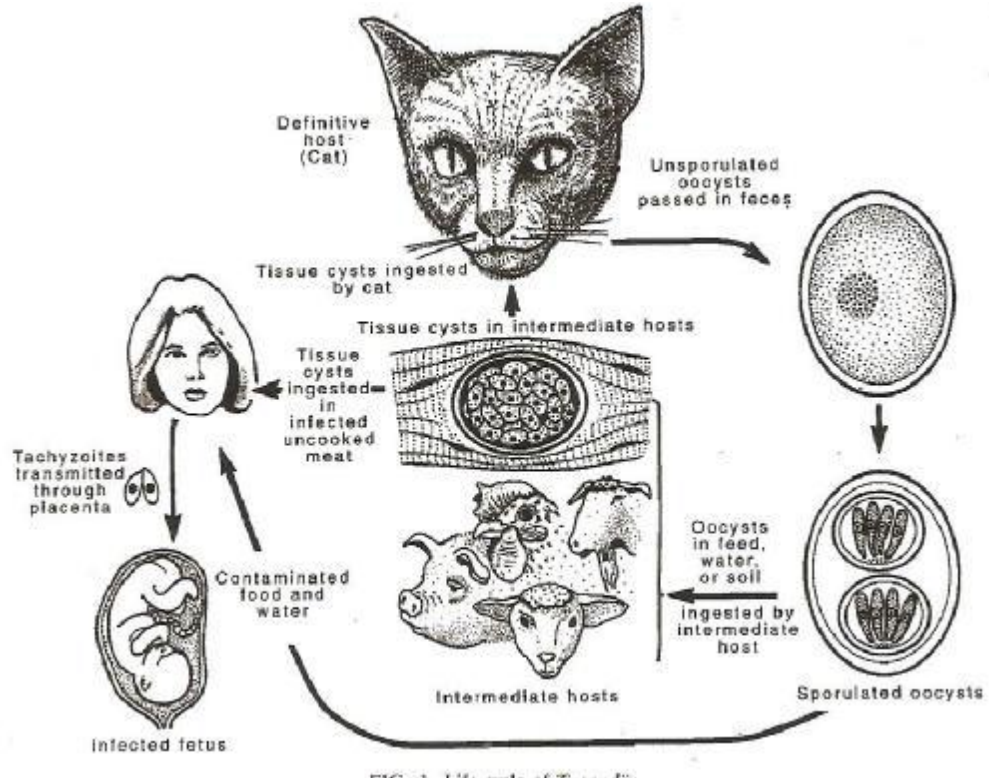

Figure 2.5 the life cycle of *Toxoplasma gondii*

Transmissions

Toxoplasmosis appears to be transmitted by:-

Congenital transmitted

1- Transplacental transmission which often leads to severe and life long disabilities in the infected infant.

Acquired transmitted

1- Ingestion of Oocyts excreated in the faces of infected cats that contaminated dust, soil and litter box material.

2- Consumption of tissue cysts under cooked or uncooked meat and certain organs of infected eggs.

3- Transmission has been known to occur by the transfusion of blood or blood products.

4- Transplacental transmission which often leads to severe and life long disabilities in the infected infant.

5- Organ transplants like kidney transplant and liver transplant.

Pathogenicity:

Unlike many bacteria and viruses, *T. gondii* actively enters the cell, in a mechanism which is mediated by the Parasites, cytoskeleton and regulated by a parasite – specific calcium – depended Secretion Pathway . The first step of cell invasion by *T.gondii* is recognition of an attachment point the two special organelles involved in this invasion Process , rhoptries and micronemes , each discharging proteins during the processes [. Following the rapid cellular invasion, the parasite resides with the a vacuole , derived primary from the host cell's plasma membrane . The active motion of *T.gondii* , called "gliding" occur with no major changes in cell shape its fast (about 10 time faster than the "crawling " rate of amoeboid cells) , and consists of both circular gliding in a counter clockwise direction and clockwise helical gliding .As an obligatory parasite , its invasive capabilities play an important role in virulence and Pathogenicity , since it can only survive intracellulary where it gets nutrients and escapes from the hosts immune response.

T.gondii invades and multiplies asexually as tachyziotes within the cytoplasm of any nucleated cell. When host immunity develops, multiplication of tachyziotes ceases and tissue cysts form which persist for years, especially in brain and muscle. Sexual reproduction of *T. gondii* occurs only in the intestinal tract of cats the resultant oocysts passed in the feceses remain infection for months . The incubation period from 10-23 days in one –common –source outbreak from ingestion of under-cooked meat and 5-20 days in an out break associated with cats.

Otherwise healthy people the infection can be asymptomatic. Symptoms may include swollen lymph nodes, a rash, malaise, fever and ‘flu’ like symptoms, and the disease is usually self-limiting. In congenital infections: disease of the new born child, or disease in later childhood or adulthood. In babies 5-10% die, 8-10% have brain and eye lesions while 10- 13% become visually impaired. Nearly all born with subclinical disease will develop symptoms later on. In other immunocompromised people disease seems to result from the activation of a previously subclinical infection. AIDS patients can experience a number of symptoms..

Laboratory Diagnosis:-

Laboratory diagnosis of acute *T.gondii* infection has historically relied on one of the following three methods:-

1- The isolation in cell culture of the tachyziote stage from blood or body fluids.

2- Demonstration of the tachyziote stage or cyst during histological examination of tissue samples (direct diagnosis method).

3- Detection of *T.gondii* antigen or specific antibody using serological method (indirect diagnosis method) .

Direct Diagnosis Method.

Histopathological Diagnosis

Demonstration of tachyziotes in tissue section or smears of body fluid (eg. CSF, amniotic fluid or BAL, placenta, peritoneal fluid, spinal cord, lymph node fluids, blood, muscles, lung, liver, bone marrow etc.) establishes the diagnosis of the acute infection it's often difficult to demonstrate tachyziotes in conventially stained tissue sections. The immunoperoxidase technique, which uses antisera to *T.gondii*, has proven both sensitive and spesific, it has been successfully used to demonstrate the presence of the parasite in the central nervous system of AIDS patients. The immunoperoxidase method is applicable to unfixed or formalin – fixed paraffin – embedded tissue sections.

A rapid and techniqually simple method is the detection of *T.gondii* in air dried, Wright Giemsa-stained slides of centrifuged (eg, Cytocentrifuge) Sediment of CSF or brain aspirate or in impression smears or biopsy tissue.

Isolation of *T.gondii*:-

Isolation of *T. gondii* from blood or body fluids establishes that the infection is acute .Attempts at isolation of the parasite can be performed by mouse inoculation.

Indirect Method :

1. ***Sabin Feldman dye test (SFDT)***

This is the first test developed for the laboratory diagnosis of *T.gondii* infection .

It is still considered the "gold standard test." SFDT detects the presence of Anti – *T.gondii* specific antibodies total immunoglobulin (total Ig).

The change in the antibody titer as determined in SFDT in consecutive serum samples taken at least 3 weeks a parts is important for the evaluation of infection during pregnancy .The tested sera are serially diluted and incubated with live tachyziotes (carrying toxoplasma –specific antigens) In the presence of separted human plasma from "Seronegative" donors (Providing) Complement complexes formed are subsequently lysed in the presence of the dye basic methylene blue (P_H 11). End – point titer is established by Counting the numbers of dead (unstained) and live (stained) parasites.

2. ***IgG avidity :-***

This is auxiliary test to determine if the infection is acute or previously acquired when the IgM serological reaction is positive in an asymptomatic patient. The test is based on the greater strength of the ionic bindings between antigen and antibody produced from old infection when compared to recent ones [45]. Depending on the method used , pregnant women with high avidity antibodies are those who have been infected at least 3-5 months earlier .This is more useful in Pregnant women in their first months of the gestation who have a positive test for both IgG and IgM Toxoplasma antibodies . When avidity is low or borderline

it may be misleading and a more careful interpretation is critical. Low – avidity results may persist for as long as 1 year .

3. ***Polymerase Chain Reaction (PCR):-***

This test is used in conjucation with standard serological test to assist in the diagnosis of *Toxoplasma gondii* infection by detection *T . gondii* DNA in tissue , blood and body fluids . Detection of *T. gondii* DNA in blood, CSF, amniotic fluid or fetal / neonatal tissue is suggestive of acute infection. Detection of *T.gondii* DNA using polymerase chain reaction (PCR) minimizes the problems faced when using serodiagnostic or culture – based assays and facilitates diagnosis in difficult cases. *T. gondii* PCR targets the *T.gondii* B1 gene, 35 copies of which are found in each organism.

While exquisitely sensitive, PCR can not distinguish between latent and acute *T. gondii* infection. Diagnosis of acute toxoplasmosis should not rely solely on the resultsof a positive PCR assay .

4. ***Enzyme Immuno assay : (EIA).***

EIA the most common laboratory tests for toxoplasmosis infection, also available as commercial kits and / or automated platforms. These tests include Enzyme linked immunosorbant assay (ELISA) and enzyme linked fluorescent immuno – assay (ELFA) which test for the presence of IgG and /or IgM antibodies specific for the parasite in human sera .EIA are useful as fasts low – cost screening tests and have been improved over the years to avoid false positive result due to non-specific detection of interfering factors such as rheumatoid factor and antinuclear antibodies.

5. ***Indirect fluorescent assay (IFA).***

The IFA was widely used to demonstrate *T.gondii* specific antibodies:

Serially diluted serum sample are incubated with live, inactivated toxoplasma fixed to a class slide. *T.gondii* specific antibodies present in the serum would bind to the inactivated parasite, and the complex is then detected using flouriscein isothiocyanate –labeled anti human Ig (or anti – IgG or anti IgM).

IFA is safer to perform and more economical than the SFDT .It appears to measure the same antibodies as the dye test, and its titer tend to parallel dye test titers. However, the IFA interpretation is subjective and time consuming. False positive results may occur with sera containing antinuclear antibodies and rheumatoid factor [49]. And false negative results of IFA for IgM may occur due to blockage by *T. gondii* - specific IgG .

6. ***Indirect Heam agglutination test (IHAT).***

As knowledge of toxoplasmosis has increase, efforts have been made to improve and extend the rang of specific diagnostic tests. In the search for alternative methods, attempts have been made to develop a haemagglutination test for the presence of toxoplasma antibodies in serum . Bozdech and jira Using sensitized

human group O Rh- negative cells, concluded that the test was less sensitive even than the complement – fixation reaction.

7. ***Complement fixation test (CFT).***

The complement fixation tests under our condition represent a classical test for the immunologic evidence of toxoplasmosis. During the 80 years of its use of recombination antigen through the modification of other components (complement, use of human erythrocytes etc) to the arrangement of the procedure . That led especially to increasing the sensitivity and specifity of the test. The significance of the classic complement fixation test for the diagnosis and evaluation of toxoplasma infection is sufficiently known .

Despite the fact that under our conditions as well as abroad the most frequent method include ELISA test for the detection of IgG antibodies , indirect Immuno fluorescent test or Sabin- Feldman dye test , despite fact that the latter is used relatively rarely due to the requirement of live strains .

Prevention **measure in pregnancy*:***

Prevention of human toxoplasmosis is based on care in avoiding the ingestion of tissue cysts and oocyst found in the environment [56]. There is no vaccine to protect humans against this disease. Prevention can be divided into primary secondary and tertiary.

Primary prevention :

When applied during pre –natal care can reduce first –time infections during pregnancy up to 63 %. This basically consists of educational and public heath programs, recommending the pregnant woman to avoid contact with material potentially contaminated with cat feces and to avoid ingestion of raw or badly-cooked meat or sub- products. The use of gloves when handing earth is also strongly recommended.

Secondary Prevention*:*

It consists of early diagnosis of the mother , the fetus and the newborn and avoiding action that can cause transplacentry transmission of the parasite , through therapeutic intervention in pregnant women and child presenting acute infection.

Tertiary Prevention:-

It's concentrated on early diagnosis through dosage of specific IgA and IgM antibodies in blood collected from the newborn allowing the use of a therapeutic regime to prevent or lower the risk of sequels .

Treatment:

The treatment of toxoplasmosis has not been a triumph of modern medicine. In the United States, there appears to be no sense of urgency since the disease is usually undiagnosed, self- limited and not considered to be a major health problems, the effectors of European workers have been more fruitful but not highly succeful.

The standard treatment in the United States, for those who required it, is limited to a combination of the anti-malarial pyrimethamine and a sulfomnamide, most commonly sulfadiazine in equal parts. The combination provides a decided synergistic effect. Both components of the regimen are capable of causing problems of toxicity and their benefits are not exciting. Careful and frequent examination of the patient, urine is required to assess the need for modification of the dose or sulfonamide used based on the appearance of crystalluria or hematuria. Because pyrimethamine is a folic acid antagonist folic acid (leucovorin calcium) is always used with it.

Trimothprim another anti material agent has not been found to be reliably effective sulfisoxazole is not effective and is not to be used. Studies of small rodents indicate a teratogenic effect of the pyrimethamine that is significantly reduced following the addition of folinic acid. Both pyrimethamine and trimothoprim have been classified as category (with regard to their level of risk for the fetus other than a questionable association with Niikawa – Kuroki Syndrome in one infant).

In most countries the favored agent for the treatment of acute Toxoplasmosis during pregnancy is spiramycin, which is not approved for use in the United States. Nevertheless, it may be obtained upon application to the Center for Disease Control in Atlanta .A single effective regimen for spiramycin treatment has not been presented although various plans have appeared. However spiramycin is freely available in India. Spiramycin is relatively a safe drug that concentrates in the placenta and may reduce the risk of maternal – fetal transmission by 60% without having any effect on the fetus.

If the fetus is shown to be infected the combination of pyrimethamine, sulfonamide (s) and folinic acid is added for the duration of the pregnancy. There are opponent of the invasive intrauterine diagnostic procedures and subsequent treatment of the elected pregnant women because they are not impressed that the regiment advocated is truly helpful.

Patients have also used combination of pyrimethamine sulfadiazine, pyrimethamine, and clindamycin for the treatment of toxoplasmosis encephalitis, and the results are comparable **.**

Antibody –functional Gold Nanorods :

Conjugate of gold nanoparticles and antibodies have useful functionalities. This test can be used to selectively target and destroy parasitic protozoans.

Gold nanorods were conjugated with anti- *Toxoplasma gondii* antibodies and used to target the extracellular tachyziote which is an infectious form of an obligate parasite *Toxoplasma gondii.*

Subsequent laser irradiation was used to kill the targeted protozoans. This concept provides a new paradigm for the treatment of parasitic protozoans.